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Antispilina ludwigi Hering, 1941 (Lepidoptera, Heliozelidae) a rare but overlooked European leaf miner of *Bistorta officinalis* (Polygonaceae): new records, redescription, biology and conservation

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Abstract. We record *Antispilina ludwigi* Hering, 1941 newly for France: Massif Central and Jura, Belgium: Ardennes and Switzerland: Jura and Alps, from many localities at middle elevations. All records were based on leafmines, often with larvae, in Snake-root, *Bistorta officinalis* Delarbre (Polygonaceae) and adults were reared from several localities. The species inhabits poor grasslands, moor habitats and heathland with relatively large hostplants. As the habitat is declining, and also other lepidopteran species feeding on this host are in decline, we expect that despite the new findings, this species is also declining and should preferably be monitored together with host specialist butterflies, such as *Boloria eunomia* (Esper, 1799) and *Lycaena helle* (Denis & Schiffermüller, 1775). During the period that the mines are present, the species is easy to record, even after the larvae have left the mines. The species is redescribed and diagnosed.

Introduction

The small leafmining family Heliozelidae has recently been the subject of several papers, including papers on phylogeny and classification (van Nieukerken and Geertsema 2015; Milla et al. 2017, 2019; van Nieukerken and Eiseman 2020), records of three new invasive species in Europe (Bernardo et al. 2011, 2015; van Nieukerken et al. 2012; Takács et al. 2020) and treatments of difficult species pairs that were previously considered single species (Mutanen et al. 2007; van Nieukerken et al. 2018). Of the thirteen known European species, nine of which are native (van Nieukerken et al. 2018; Takács et al. 2020), the small *Antispilina ludwigi* Hering, 1941, the only one feeding on a herbaceous plant, remains relatively poorly known.

Hering (1941) described the species and genus on the basis of moths reared from leafmines on Snake-root (or Common Bistort), *Bistorta officinalis* Delarbre (also known as *Polygonum bistorta* L. or *Persicaria bistorta* (L.) Samp.), collected from Hessen, Germany by A. Ludwig, a record several times repeated in literature (Grabe 1944; Skala 1949; Ludwig 1952). After that there have been only a few published faunistic records based on leafmines from Germany: Nordrhein-Westfalen (Ludwig 1952), Sachsen (Buhr 1964), Poland (Borkowski 2003b; Buszko and Pałka 2016), the

Czech Republic (Liška et al. 2000) and Lithuania (Ivinskis 2004), all with relatively little information. Several of these records have been overlooked subsequently, e.g. the German checklists did not cite it for Sachsen (Gaedike and Heinicke 1999; Gaedike et al. 2017), but the first version did cite Baden-Württemberg, a record later removed as no original sources could be found (Gaedike 2010).

When working on the family Heliozelidae the first author received ample material from Poland for molecular work. He then realised that it should be possible to find the species in other European mountains by focused searching of the hostplant for mines. In 2009 he encountered many mines during a hike on the Mont Mézenc in the Massif Central of France. This success led to a more focussed search in July 2017 in Auvergne and prompted him to encourage SW and RB to search for *Antispilina ludwigi* in the Belgian and Swiss mountains. To our surprise we all succeeded, and here formally record the species as new for Belgium, France and Switzerland. The records for Belgium and Switzerland were already available in a rather cryptic form online (Lepiforum e.V. 2008–2021; Wullaert 2021), and material from France and Belgium was used in our published DNA barcode and phylogenetic analyses (van Nieukerken et al. 2012; van Nieukerken and Geertsema 2015; Milla et al. 2017, 2019).

We redescribe the species, for which no full descriptions were available, provide information on its biology, and discuss conservation issues concerning this rare but overlooked insect.

Material and methods

Collecting

Most of the material treated here was collected by three of the authors as larvae and leafmines. Leafmines were searched by checking the hostplants. Samples of leaves with mines were taken home in plastic bags. Where needed some larvae were removed from their mines for molecular and morphological study and stored in 96% ethanol. Samples of leafmines were dried in a plant press and are stored in glassine envelopes. Additionally, larvae from Poland were sent to EvN by Anna Mazurkiewicz as preserved specimens in ethanol 96% in individual vials.

Rearing

Collected leaves were kept in small jars or polyethylene bags, with some moss and/or paper tissue added, until the larvae had prepared their leaf-epidermis-shield cases in the fourth instar. It was often necessary to remove the cut-out cases manually from the leaves, when they failed to detach probably due to loss of turgor in the leaves, after which the leaves were taken from the rearing jars and dried as vouchers. Adults were reared after hibernation under low temperatures, either in a fridge at ca. 4° C (French material in Leiden) or outdoors (Switzerland). Adults were reared successfully after the containers were taken indoors in early March (EvN) or left out of doors under natural conditions (RB). Larvae brought indoors on 29 January in Switzerland failed to pupate and died prematurely. However, a few parasitoids did emerge from these (see below). No adults emerged from Belgian larvae that were kept in a cool room.

Material

In Leiden all larval specimens, samples of leafmines and the majority of adults received a registry number in the form RMNH.INS.#####. As all material was either reared from leafmining larvae or collected as leafmines, all on the same hostplant *Bistorta officinalis*, we do not cite the hostplant

in the material lists. Numbers of larvae and mines for Belgian collection records refer to observed numbers, of which a sample was taken for rearing (that unfortunately failed) and some mines are stored in the collection.

We list only observations for new records, not those that duplicate the collections records. A detailed dataset of material, all observations and literature records was uploaded via NLBIF to GBIF (https://doi.org/10.15468/be5dvu).

To find additional records, hundreds of photographs of live and herbarium specimens of *Bistorta officinalis* were examined from several web portals (https://bioportal.naturalis.nl/; http://www.botanicalcollections.be/#/en/home; http://vh.gbif.de/vh/static/en_startpage.html; https://www.gbif.org/). This, however, did not provide any additional records.

Abbreviations

MfN Museum für Naturkunde Berlin, Leibniz-Institut für Evolutions und Biodiversitätsforschung an der Humboldt-Universität, Berlin;

RBC Bryner, R., personal collection, Biel, Switzerland;

RMNH Naturalis Biodiversity Center, Leiden, Netherlands;

SWC Wullaert, S., personal collection, Houthalen, Belgium;

WB Werkgroep Bladmineerders [leafmine working group Belgium].

Morphology

Methods for preparation of the genitalia, other body parts and larval pelts follow our earlier work (van Nieukerken et al. 2012, 2018). Morphological terminology for adults and larvae follows recent Heliozelidae treatments (van Nieukerken and Geertsema 2015; van Nieukerken et al. 2018; van Nieukerken and Eiseman 2020).

Illustrations

Photographs of moths, leafmines, genitalia slides and larval slides in Leiden were taken with a Zeiss AxioCam digital camera attached, respectively, to a Zeiss Stemi SV11 stereo-microscope, a motorized Zeiss SteREO Discovery.V20 or a Zeiss Axioskop H, using Carl Zeiss AxioVision software version 4.8 or 4.9. Full depth views of male genitalia were made by stacking individual photographs with Zerene Stacker 1.04.

Field photographs by EvN were taken with a Canon EOS 350D or EOS 600D, all photographs by RB were taken with a Canon EOS 5D, Mark II with a Canon MP-E 65mm lens and those by SW with a Nikon D5100, AF-S Micro Nikkor 105mm lens.

Line drawings were prepared by BWL from genitalia in glycerin, using pieces of tissue and pins to fix the parts in position under the microscope, while the drawing was prepared.

The distribution map was prepared with QGIS 3.10.14.

DNA barcoding

All nine available DNA barcodes were generated for our earlier studies, and six of these were published (van Nieukerken et al. 2012, 2018; Milla et al. 2017, 2019). Three more are here presented for the first time. All data, including GenBank accession numbers are available in Barcoding of Life Dataset DS-ANTILUDWI (https://doi.org/10.5883/DS-ANTILUDW). Procedures are detailed in the cited papers.

Taxonomy

Antispilina ludwigi Hering

Common names: Knöterich-Erzglanzfalter (German), Adderwortelgaatjesmaker (Dutch)

Antispilina ludwigi Hering, 1941:19. Holotype ♂, **Germany**: [Hessen] "Dillkreis, Im Feuerhack bei Waldaubach", emerged 20 March 1941. Mines 29 July 1940, *Polygonum bistorta* [MfN] [examined]

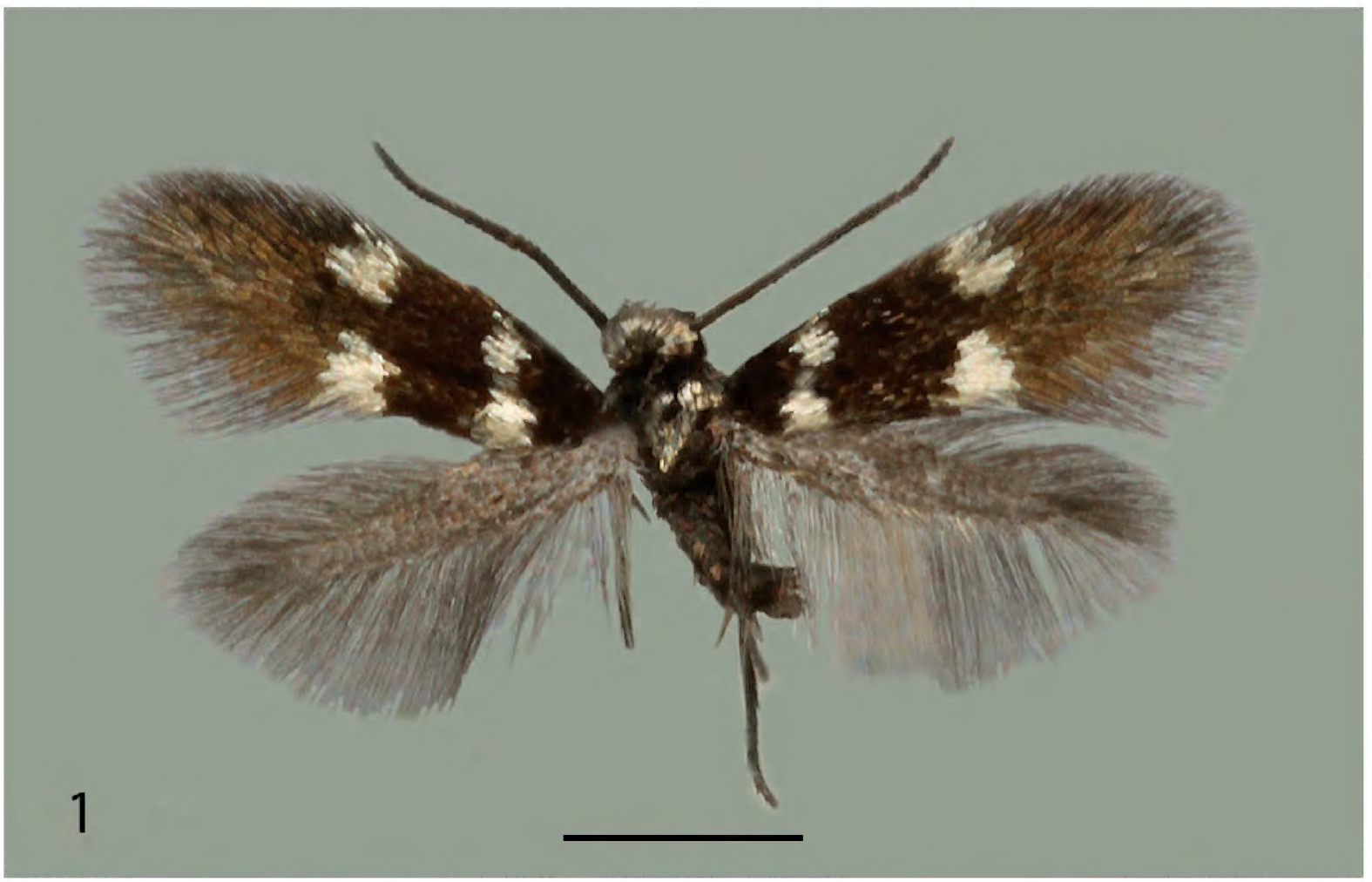
Antispilina ludwigi; Ludwig 1952: 24; Grabe 1955: 100; Buhr 1964: 37 [Sachsen]; Wojtusiak 1976: 9 [Poland, key, description, genitalia + adult ill.]; Razowski 1978: 91. [Poland, redescription, ill]; Laštůvka 1998: 17 [Czech Republic, checklist]; Gaedike and Heinicke 1999: 43 [Germany, catalogue]; Liška et al. 2000: [Czech Republic]; Borkowski 2003a: 88 [Poland]; Borkowski 2003b: 113 [Poland, photographs adults and mines]; Ivinskis 2004: 36 [Lithuania]; Buszko and Pałka 2016: 245 [Poland]; Gaedike et al. 2017: 24 [Germany, catalogue]; Buszko and Nowacki 2017: 17 [Poland, catalogue]; van Nieukerken 2018: https://fauna-eu.org/cdm_dataportal/taxon/6b71a06e-5ecb-48b6–84f0–45462a9d4604 [Europe, catalogue]; Laštůvka et al. 2018: 121–122 [Czech Republic, colour illustrations]; Bryner in Lepiforum 2003–2021: http://www.lepiforum.de/lepiwiki.pl?Antispilina_Ludwigi [Switzerland, photographs all stages, genitalia and mines]; Werkgroep Bladmineerders 2020: http://www.bladmineerders.be/nl/content/antispilina-ludwigi-m-hering-1941 [Belgium, photographs mines, larvae], De Prins and Steeman 2003–2021: https://projects.biodiversity.be/lepidoptera/species/5448/ [Belgium, catalogue].

Diagnosis. *Antispilina ludwigi* is a small completely dark grey-black moth, including the forewing fringe, with a narrow silver fascia at one-third and two opposite silver spots at two-thirds, resembling *Antispila* species. The small size (4–5 mm wingspan) and the dark colour separate *A. ludwigi* from the larger *Antispila* species (4.8–9 mm), that also have a distinct fringe line and white forewing fringe. *Holocacista rivillei* (Stainton, 1855) is about the same size, but the fascia is usually divided, it has also a white fringe, and the male foretibia bears a small epiphysis and it is not so dark. The latter species occurs in Mediterranean habitats with wild or cultivated grapevine (*Vitis vinifera* L.), whereas *A. ludwigi* is confined to poor grasslands and moors, mostly in mountains. A key was provided by van Nieukerken et al. (2018). The leafmine is the only blotch mine known on *Bistorta officinalis*, but early mines may sometimes be confused with the reddish circular patches caused by the fungus *Ramularia bistortae* Fuckel (Mycosphaerellaceae, Ascomycota) (Ellis 2020) (e.g. Fig. 26).

Description. Male (Figs 1, 24, 25). Forewing length 2.1–2.4 mm, wingspan ca. 4.5–5.0 mm. Head (Figs 3–5): frons with smooth scaling, bronzy-fuscous with brassy reflection; vertex bronzy-fuscous. Antenna reaching 2/3 of forewing, with 18–19 segments, basally covered with fuscous scales; scape pale brown ventrally with pecten consisting of 3 or 4 hairs. Labial palpus pale fuscous, comprising 2 palpomeres; maxillar palpi minute, 1-articled. Haustellum as long as head capsule. Thorax and tegulae brassy with metallic reflections, may appear silvery in some lighting. Legs dark grey, no white spots, epiphysis absent. Forewing almost jet-black, apically with brassy reflections, a silvery white, narrow outwardly oblique fascia at 1/5 to 1/4, usually constricted in middle; a silvery white small triangular dorsal spot at 1/2, and a squarish costal spot slightly beyond dorsal one; fringe grey with brassy reflection, fringe line absent. Hindwing and fringe dark grey. Abdomen greyish black.

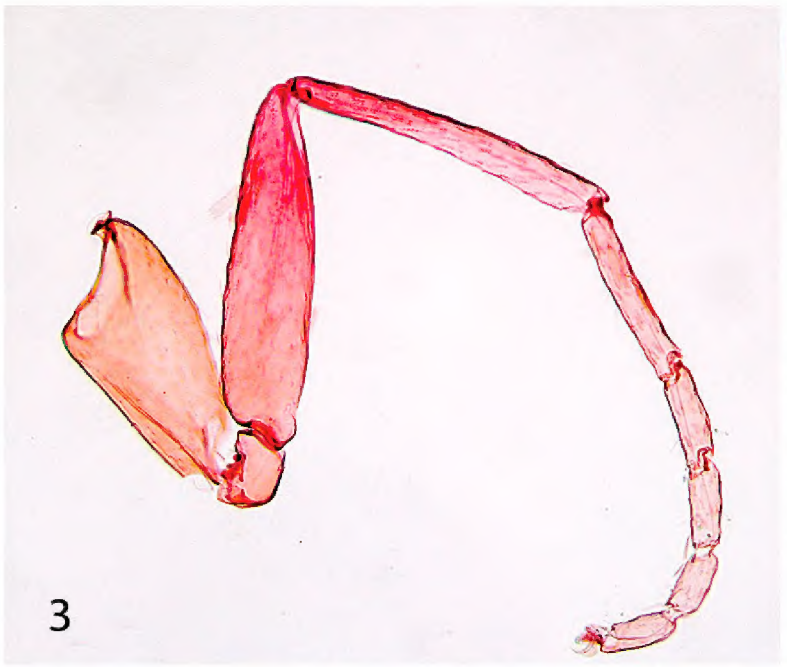
Female (Fig. 2). Forewing length 2.0–2.3 mm, wingspan ca. 4.2–5.0 mm. Abdomen apically pointed.

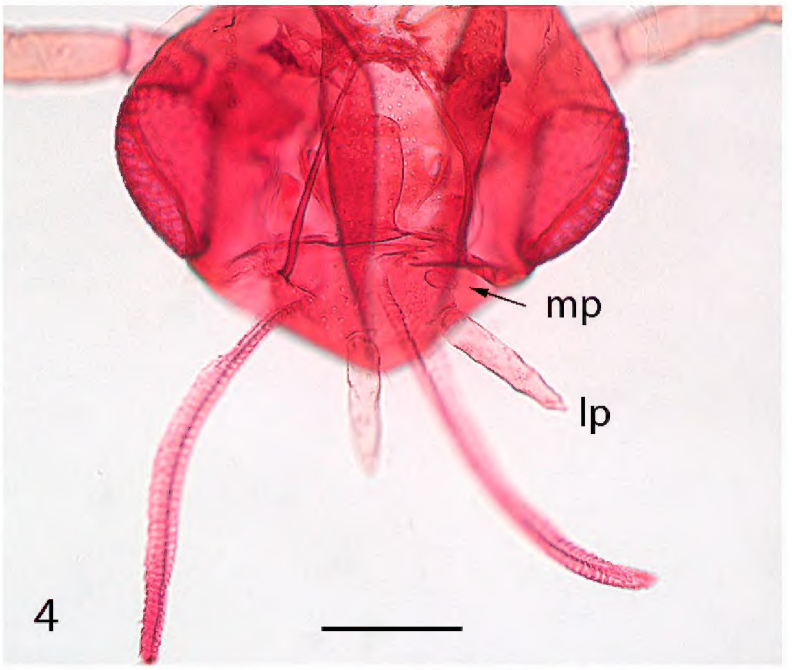
Venation (Fig. 6). Forewing: Sc poorly visible. R unbranched, a separate vein, to costa, but a persistent trachea connecting R with Rs+M+CuA. Rs+M+CuA ending in 4 rather indistinct branches, Rs1+2 to costa, Rs3+4 to termen, M and CuA to dorsum. Hindwing with Sc+R to costa, poorly visible, Rs+M with 3 branches, Rs indistinct, to costa, 2 branches of M to termen and dorsum; CuA a separate vein to dorsum; no visible Anal vein.

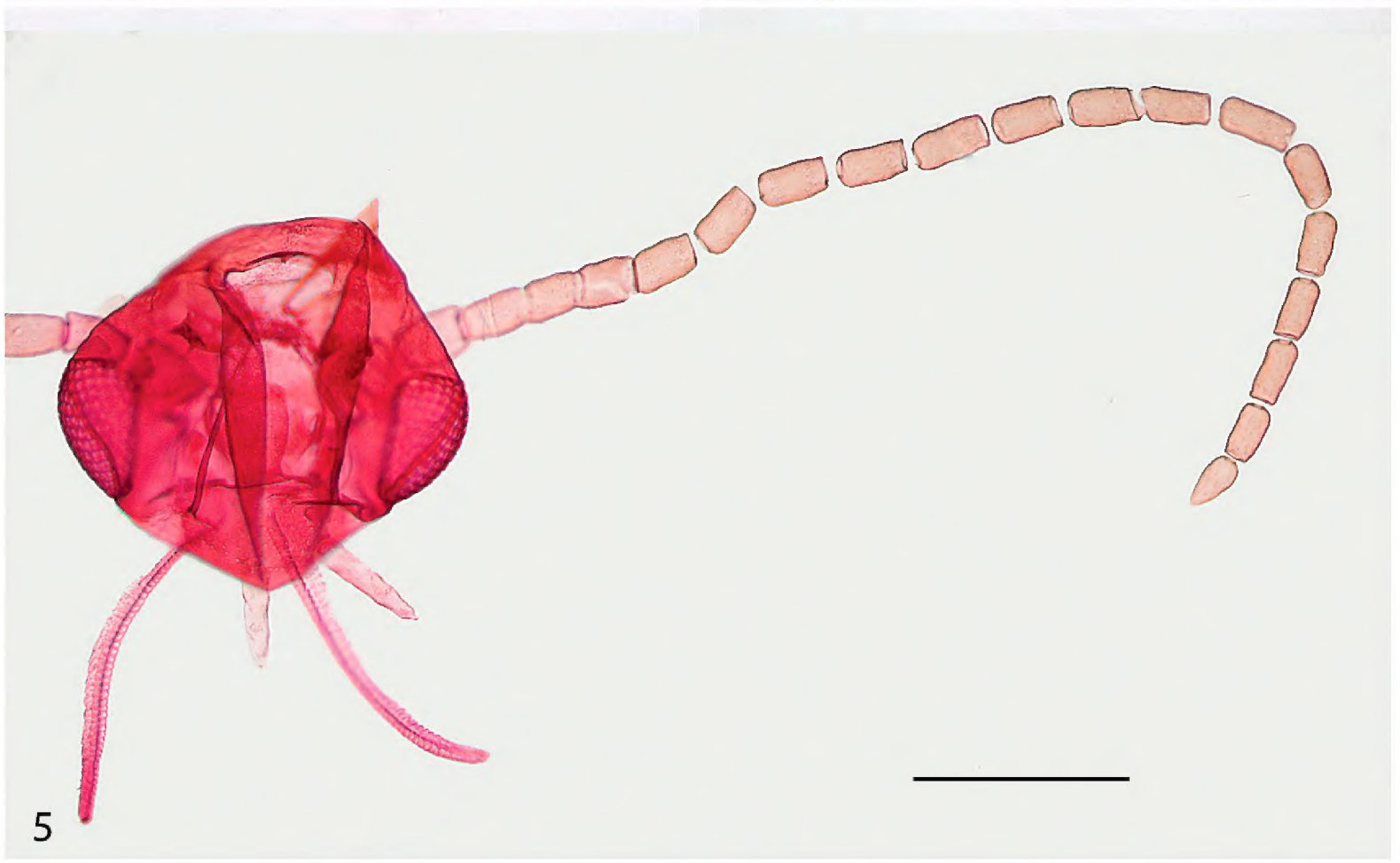


Figures 1, 2. *Antispilina ludwigi*, male and female. Switzerland, Bern, Rüeggisberg. Scale bar 1 mm. Photographs R. Bryner.

Male genitalia (Figs 7–13). Total length ca 530 µm. Vinculum well sclerotized, long, narrowest in middle, as long as valva or longer. Tegumen bilobed. Valva subtriangular with blunt apex, length twice width, stalked pecten ca. 2/3 from base of valva, with ca. 12–13 blunt sensilla, the number







Figures 3–5. *Antispilina ludwigi*, male adult, morphological details, whole body slide EvN4448. **3.** Foreleg. **4**. Head and mouthparts, lp: labial palp with 2 palpomeres; mp: maxillary palp, 1-articled. **5.** Head and antenna with 19 segments. Scale bars: 100 µm (1, 2), 200 µm (3). Photographs E.J. van Nieukerken.

on each valva may differ. Transtilla with long sublateral processes and slightly bilobed posterior process. Juxta weakly sclerotized, three-pronged anteriorly. Phallus cylindrical, ca. 400 µm long, without cornuti, distal part slightly swollen, about as long as vinculum; phallocrypt spinose.

Female genitalia (Figs 14–17). Tip of oviscapt dorsoventrally flattened, with five cusps in total. Posterior apophyses slightly longer than anterior apophyses. Eighth sternum membranous, except narrow sclerotized region in distal part. Eighth tergite sclerotized, posterior part dorsally elevated, anterior end crooked, middle part projected anteriorly. Guy wire arising from middle part of vestibulum, as long as vestibulum. Vestibulum membranous with a pair of sclerotized rods.

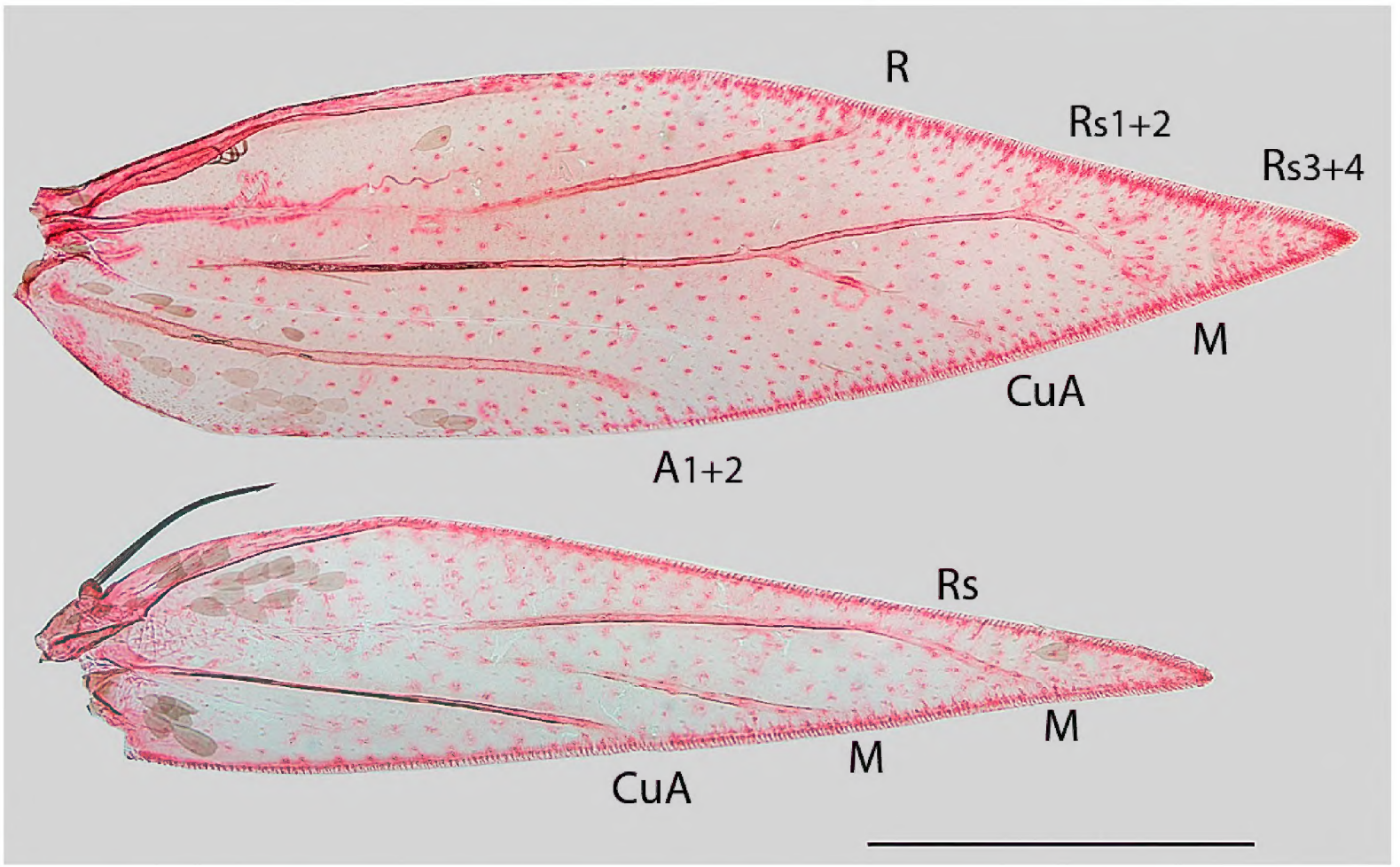
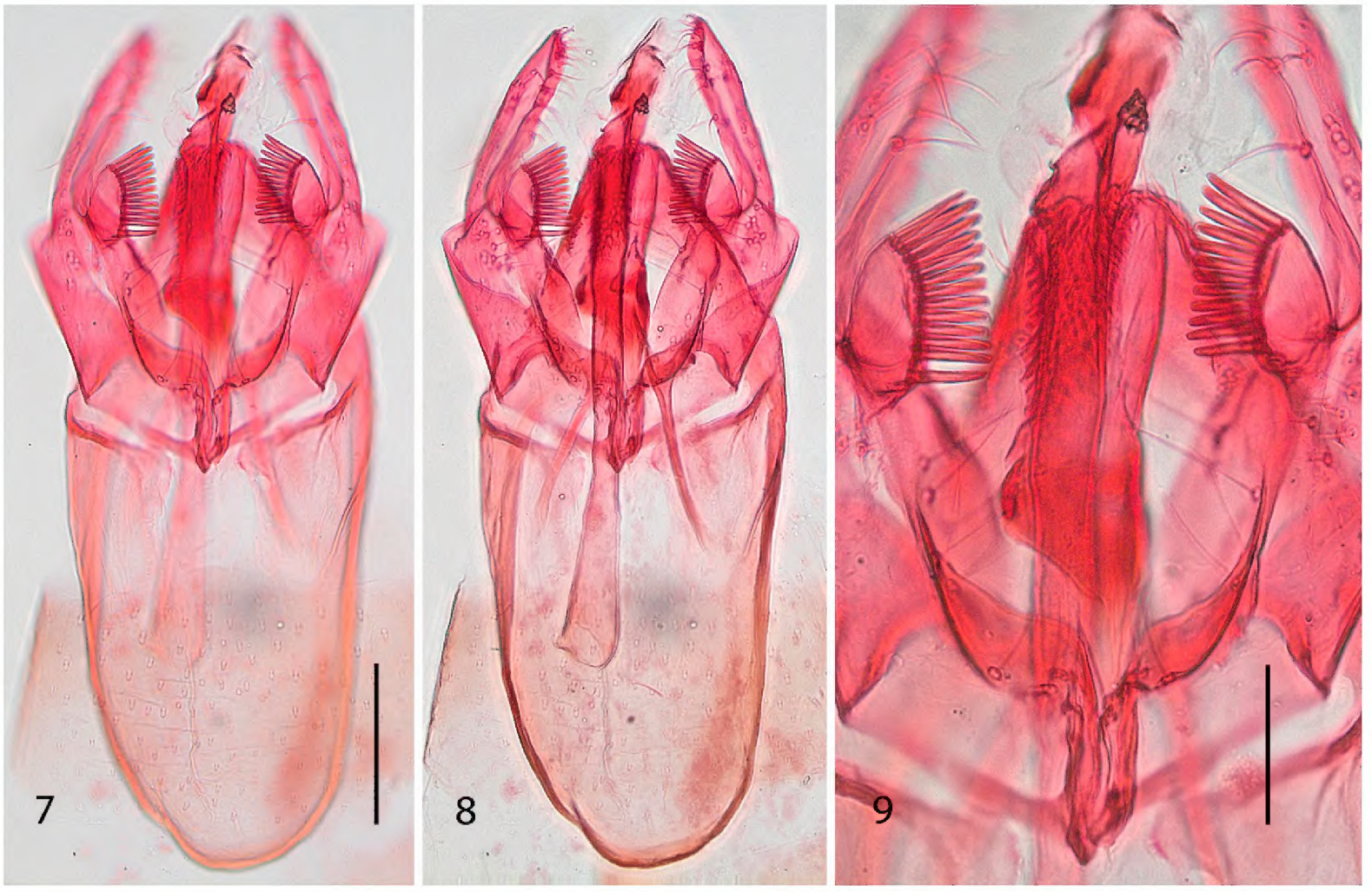
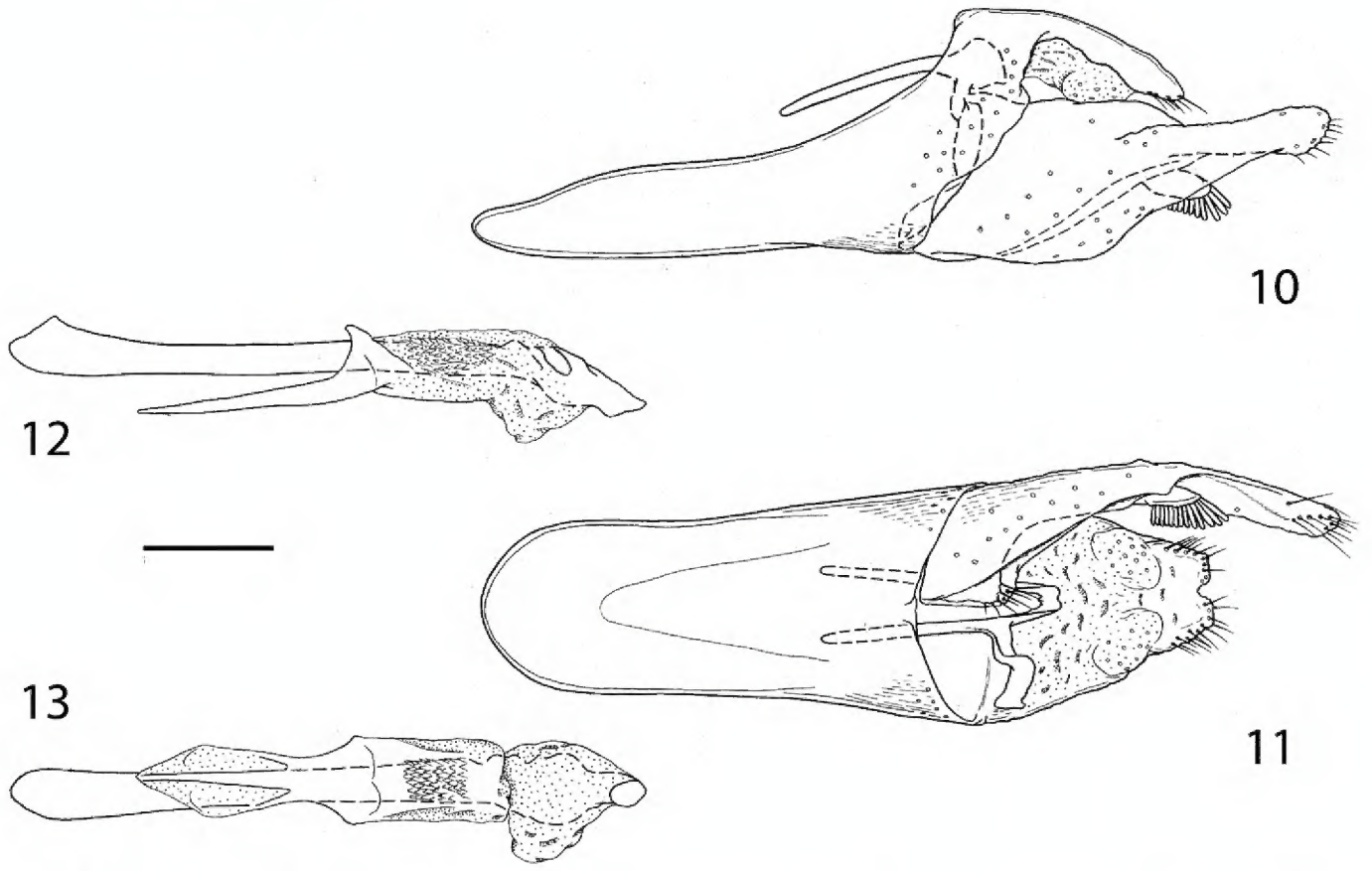


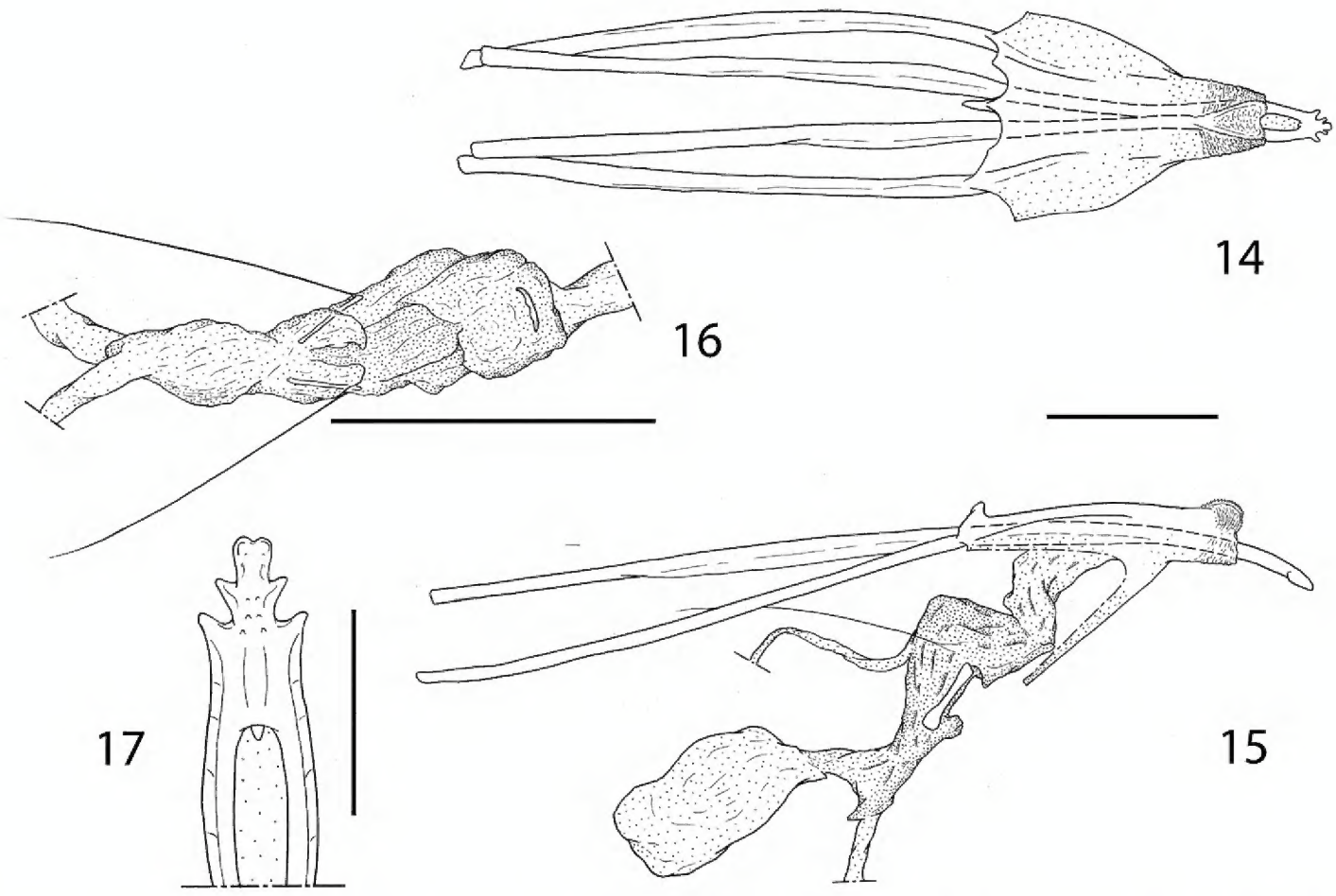
Figure 6. *Antispilina ludwigi*, male venation, slide EvN4676. Photograph E.J. van Nieukerken.



Figures 7–9. *Antispilina ludwigi*, male genitalia, slide EvN4448. **7.** Ventral view, single exposure. **8.** Stacked image from four individual exposures. **9.** Detail, stack from two exposures. Scale bars 100 µm (7, 8), 50 µm (9). Photographs E.J. van Nieukerken.



Figures 10–13. *Antispilina ludwigi*, male genitalia, Germany, Hessen: Dillkreis. Drawn in glycerin. **10, 11.** whole genitalia, phallus removed, lateral and ventral view. **12, 13.** phallus, lateral and ventral view. Scale bar 100 µm. Art work Bong-Woo Lee.



Figures 14–17. *Antispilina ludwigi*, female genitalia, Germany, Hessen: Dillkreis. Drawn in glycerin. **14, 15.** whole genitalia, lateral and ventral view. **16.** vestibulum, ventral view. **17.** Oviscapt, detail. Scale bars 200 µm, 50 µm (17). Art work Bong-Woo Lee.

Larva (Figs 18–22, 35). Body white, prothoracic plates and head pale brown. Prosternum and pronotum with ovate plates (sclerites) in what we assume are the 4th and 5th instars (see van Nieukerken and Eiseman 2020); earlier instars not examined in detail), other segments and prothorax outside the plates as well covered with small transverse swellings. Head width 4th instar ca 400 µm. Legs and prolegs absent. The 4th instar cuts out the flat cocoon or shield from pieces of the leafmine.

Pupa (Fig. 23). Not studied in detail.

Biology. Host plant. Snake-root (or Common Bistort), *Bistorta officinalis* Delarbre (= *Polygonum bistorta* L., *Persicaria bistorta* (L.) Samp., *Bistorta major* Gray) (Polygonaceae).

Leafmine (Figs 26–35). The exact oviposition site has not been determined, but in most cases this must be close to the midrib (87% of the French mines, n=251), or close to another large vein. The larva first makes a circular blotch close to the vein with the frass glued to the upper and under epidermis, resulting in a round red-brownish spot on both leaf surfaces. From there the mine enlarges into an elongated blotch mine, usually between lateral veins towards leaf margin; the blotch is further transparent and whitish in colour. From there the frass is more scattered, blackish, partly in clumps and no longer concentrated throughout the rest of the mine. The outer edges of the mine are irregularly eaten out and do not contain any frass. In many cases the mine is not larger than ca 1 cm, in thin leaves this can be larger. At the end the caterpillar returns to the round spot and makes there an elongate to circular leaf cut from both epidermal layers (reinforced by the frass) of ca. 3 mm long (Fig. 32). There are often several mines per leaf, and mines regularly occur as "twins" at both sides of the midrib (e.g. Fig. 31). In some cases several mines converge into large communal mines, where several larvae may feed next to each other (Fig. 35). Leaves with many mines are very conspicuous, even from a distance.

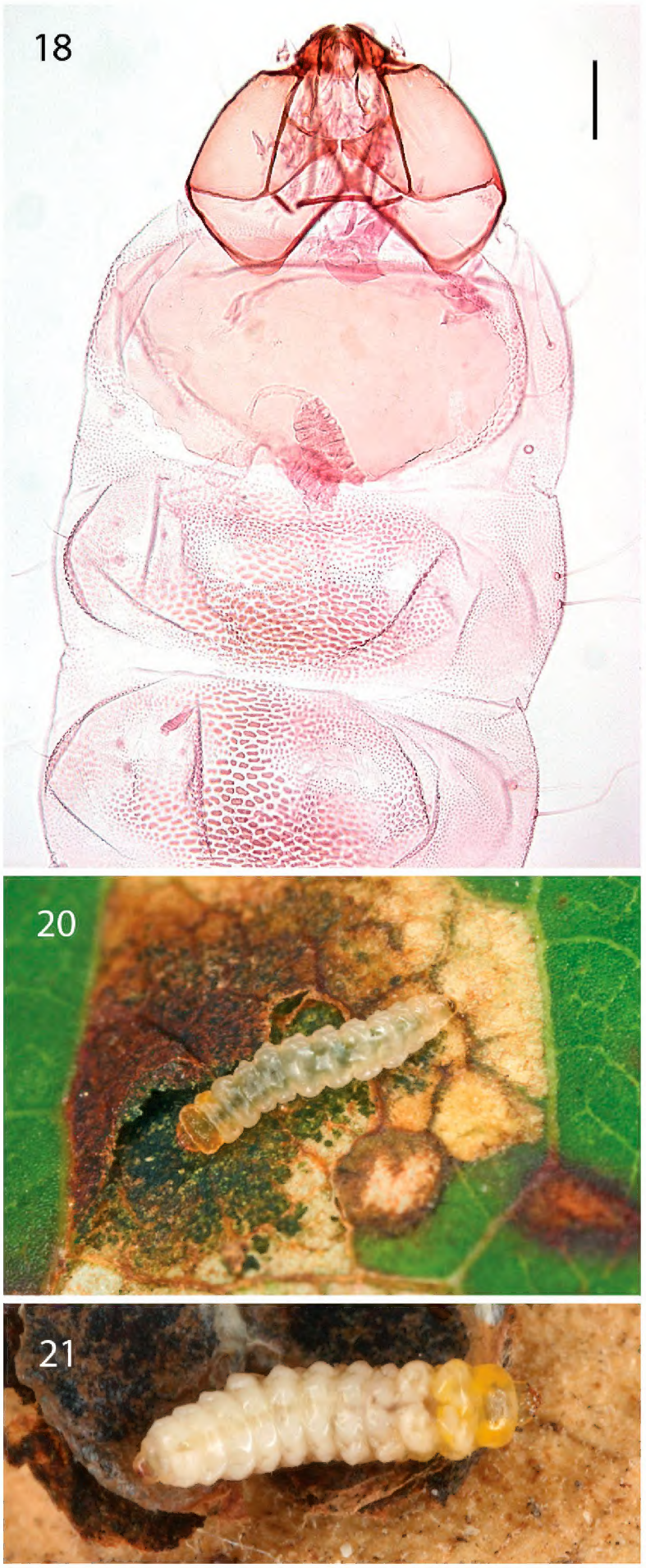
The larva crawls away with the shield (Fig. 22) and seeks a shelter, where it will moult inside the shield into the non-feeding 5th instar and later pupate. The larva probably attaches the shield to some plant material (Fig. 36) as in the other genera in the *Holocacista*-group (van Nieukerken and Eiseman 2020).

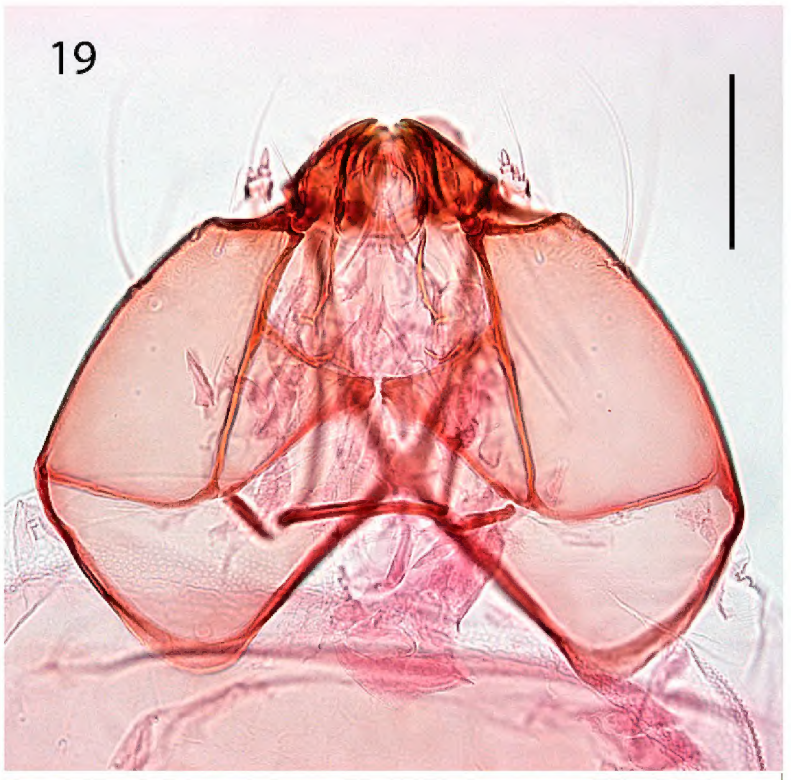
Larvae were found from June to September (own data; Liška et al. 2000; Borkowski 2003b; Buszko and Pałka 2016), earlier at lower altitudes, in the mountains most larvae were active in July. Reared adults always emerged after hibernation the next spring between March and June; one adult was swept from the hostplant on 27.v.2020 in the locality St. Imier at 1095 m (Switzerland). We assume that there is only one generation, with an extended period of larval feeding, and adults flying from April to June depending on altitude.

Parasitoids. We only reared three specimens of *Pnigalio* sp. (Eulophidae, identified by Hannes Baur) and one Chalcidoidea, unidentified to lower taxon (identified by Seraina Klopfstein). All emerged in February from cocoons that were taken indoors in January (locality Saignelégier, Jura, Switzerland). No parasitoids emerged from the extensive breedings of French material.

Distribution (Fig. 40). Central Europe: recorded from Belgium, France, Switzerland , Germany, the Czech Republic, Poland and Lithuania.

The species has previously only been found a few times: in Germany in a small area in Hessen and Nordrhein-Westfalen (Hering 1941; Ludwig 1952; Grabe 1955) and Sachsen (Buhr 1964); in Poland it was known from the Sudety mountains (Wojtusiak 1976; Razowski 1978; Buszko and Nowacki 2000; Borkowski 2003a, b), but later also found in the lowlands in the South-East (Buszko and Pałka 2016), one record from the Czech Republic, also from the Sudety range (Liška et al. 2000) and from southern Lithuania (Ivinskis 2004). Here we report its occurrence for the first time from France (Mas-





Figures 18–23. *Antispilina ludwigi*, immature stages. **18, 19.** Details of slide mounted caterpillar, probably 4th instar, Poland, RMNH.INS.12423; scale bars 100 µm. **20.** Caterpillar, final feeding instar (4th) in opened mine, Switzerland, St-Imier, 2.viii.2017. **21.** Non feeding instar (5th) from opened shield, Switzerland, Saignelégier, 5.ii.2018. **22.** Caterpillar walking with shield, Switzerland, St-Imier, 3.viii.2017. **23.** Pupa in opened cocoon, Switzerland, Saignelégier, 4.v.2018. Photographs E.J. van Nieukerken (18, 19), R. Bryner.

Figures 24–25. *Antispilina ludwigi*, live male, Switzerland, Saignelégier, 4.v.2018. Photographs R. Bryner.

Figures 26–33. *Antispilina ludwigi*, leafmines and larvae on *Bistorta officinalis*. **26, 27.** France, Mt. Mézenc, 29.vii.2009. **28, 29.** France, Puy-de-Dôme, Res. Nat. Chastreix-Sancy, resp. Roc de Courlande and Fontaine Salée, 13.vii.2017. **30–33.** Belgium, Rocherath, Naturschutzgebiet der Holzwarche, 23.vi.2017. Photographs E.J. van Nieukerken (26–29), S. Wullaert (30–33).

Figures 34–37. *Antispilina ludwigi*, leafmines and larvae on *Bistorta officinalis* and habitat. **34–36.** Switzerland, Saignelégier, 4.viii.2017. **37.** Habitat in Switzerland, Jura, St-Imier, probably in flying time of *Antispilina ludwigi*, taken 21.vi.2009. Photographs R. Bryner.

sif Central: Auvergne, Cevennes and Jura), Switzerland (Jura and Alps) and Belgium (Ardennes). It is very likely that *A. ludwigi* also occurs further east in Belarus, Ukraine or Russia, where Microlepidoptera and especially leafmining Lepidoptera have only been collected very sparsely.

Habitat (Figs 37–39). Most of our own records were obtained in mountainous bogs, poor wet meadows, often along streams and montane heathland where the host is abundant. This is often patchy habitat with shrubs and sometimes scattered larger trees. The attacked hostplants often are relatively large and leaves with mines are usually hidden among the vegetation. Smaller plants in mowed grasslands rarely had mines. The following plants were common in many of the localities in the Massif Centrale: *Arnica montana* L., *Betula pubescens* Ehrh., *Calluna vulgaris* (L.) Hull, *Cicerbita alpina* (L.) Wallr., *Cytisus oromediterraneus* Rivas Mart. et al., *Dianthus gratianopolitanus* Vill., *Genista pilosa* L., *G. tinctoria* L., *Gentiana lutea* L., *G. pneumonanthe* L., *Meum athamanticum* Jacq., *Potentilla erecta* (L.) Raeusch., *Rumex acetosa* L., *Sanguisorba officinalis* L., *Succisa pratensis* Moench, *Trollius europaeus* L., *Vaccinium myrtillus* L., and *V. uliginosum* L.

Buszko and Pałka (2016) found the species in lowland habitats in '*Molinietum-caeruleae*' meadows, between 170 and 215 m. Our records are from mountains between 450 and 1650 m elevation and also most published records are from mountains in similar elevational range.

DNA barcodes. Nine specimens were barcoded, representing populations in Poland, France and Belgium. All cluster within Barcode Identification Number BOLD:AAW5935, with an average distance of 0.36% and a maximum distance of 0.81%. (BOLD:AAW5935; dataset https://doi.org/10.5883/DS-ANTILUDW). The nearest neighbour, at a distance of 9.38%, is an unnamed heliozelid from Costa Rica.

Remark. There are several specimens in collections with paratype labels, such as the three cited here (and see Lepiforum e.V. (2008–2021)), but as Hering (1941) cited a single specimen in the description, the type material consists only of a holotype, and no paratypes.

Material examined. 21 ♂♂ 19 ♀♀, cocoons. All specimens are reared from *Bistorta officinalis*, except where indicated.

FRANCE • 2 ♂♂ 1 ♀; Ardèche, Mt Mézenc, SW slopes; 44.90835°N, 4.18781°E; alt. 1652 m; 29.vii.2009; E.J. van Nieukerken leg.; emerged 03.iv– 03.v.2010; EventId: EvN no 2009032-K; Genitalia slides: EvN4448 ♂ (whole body slide), EvN4676 ♂, EvN4677 ♀; RMNH.INS.24448, RMNH.INS.24676, RMNH.INS.24677. • 5 ♂♂ 4 ♀♀, cocoons; same data; emerged 03.iv– 06.v.2010; no registry numbers; RMNH. • 2 cocoons, 1 with prepupa; Ardèche, Sainte-Eulalie, Bois des Seigneurs; 44.86449°N, 4.1827°E; alt. 1455 m; 23.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017053-K; RMNH.INS.16908.• 4 ♂♂, 10 cocoons (plus exuviae); Ardèche, Sainte-Eulalie, Suc de la Lauzière, S.; 44.84695°N, 4.17325°E; alt. 1483 m; 23.vii.2017; E.J. van Nieukerken leg.; emerged 02–22.iv.2018; EventId: EvN no 2017051-K; RMNH.INS.16584–RMNH.INS.16587, RMNH.INS.16907. • 2 ♀♀; Puy-de-Dôme, Monts Dore, Chastreix-Sancy, Roc de Courlande, S.; 45.52231°N, 2.7688°E; alt. 1339 m; 13.vii.2017; E.J. van Nieukerken leg.; emerged 02.iv.2018; EventId: EvN no 2017034-K; RMNH.INS.16567–RMNH.INS.16568. • 2 ♂♂ 4 ♀♀ 1 ad, 10 cocoons (plus exuviae); Puy-de-Dôme, Monts Dore, Chastreix-Sancy, Res. Nat, Fontaine Salée; 45.51214°N, 2.79792°E; alt. 1338 m; 13.vii.2017; E.J. van Nieukerken leg.; emerged 29.iii– 09.iv.2018; EventId: EvN no 2017035-K; RMNH.INS.16569–RMNH.INS.16575, RMNH.INS.16902. • 3 ♂♂; Puy-de-Dôme, Monts Dore, Col de la Croix de St Robert, SE, along road; 45.55873°N, 2.84282°E; alt. 1416 m; 16.vii.2017; E.J. van Nieukerken leg.; emerged 03–12.iv.2018; EventId: EvN no 2017039-K; RMNH.INS.16578–RMNH.INS.16580. • 1 ♂; Puy-de-Dôme, Monts Dore, Vallée de Chaudefour, Res. Nat., upper valley E; 45.52916°N, 2.83496°E; alt. 1346 m; 16.vii.2017; E.J. van Nieukerken leg.; emerged 04.iv.2018; EventId: EvN no 2017037-K; RMNH.INS.16577. • 4 cocoons; Puy-de-Dôme, Monts Dore, Chastreix-Sancy, Roc de Courlande, S.; 45.52231°N, 2.7688°E; alt. 1339 m; 13.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017034-K; RMNH.INS.16901. • 9 cocoons; Puy-de-

Figures 38, 39. Habitats of *Antispilina ludwigi*. **38** (top). Belgium, Rocherath, Naturschutzgebiet der Holzwarche, 24.vi.2017. **39** (bottom). Ardèche, Sainte-Eulalie, Suc de la Lauzière, 23.vii.2017. Photographs: A. Rauw (38), E.J. van Nieukerken (39).

Figure 40. Distribution of records of *Antispilina ludwigi*, including all literature records.

Dôme, Monts Dore, Col de la Croix de St Robert, SE, along road; 45.55873°N, 2.84282°E; alt. 1416 m; 16.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017039-K; RMNH.INS.16905. • 1 cocoon; Puy-de-Dôme, Monts Dore, Vallée de Chaudefour, Res. Nat., upper valley E; 45.52916°N, 2.83496°E; alt. 1346 m; 16.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017037-K; RMNH.INS.16904.• 1 ♀, 5 cocoons (plus exuviae); Puy-de-Dôme, St. Alyre-ès-Montagne, Lac de St. Alyre, S. shore; 45.37424°N, 2.94417°E; alt. 1224 m; 14.vii.2017; E.J. van Nieukerken leg.; emerged 09.iv.2018; EventId: EvN no 2017036-K; RMNH.INS.16576, RMNH.INS.16903.

GERMANY • 2 ♂ 1 ♀ [labelled as paratypes]; Hessen, Dillkreis, Waldaubach, im Feuerhack; 50.666474°N, 8.132751°E; alt. 570 m; 29.vii.1940; A. Ludwig leg.; emerged 10.iii.1941; Genitalia in vial; MfN.

SWITZERLAND • 1 ♂ 1 ♀; Bern, Rüeggisberg; 46.7307°N, 7.45849°E; alt. 1400 m; 07.viii.2017; R. Bryner leg.; emerged 04–06.v.2018; Genitalia slide ♂ 2018–031; RBC. • 2 ♀♀; Bern, St-Imier; 47.12879°N, 6.98725°E; alt. 1095 m; 02.viii.2017; R. Bryner leg.; emerged 04–08.v.2018; RBC. • 1 ♀; same locality; 27.v.2020; R. Bryner & D. Bolt leg.; day catch; collection Daniel Bolt, Domat/Ems.• 1 ♂ 2 ♀♀; Jura, Saignelégier; 47.23961°N, 7.04089°E; alt. 1000 m; 04.viii.2017; R. Bryner leg.; emerged 04–12.v.2018; Genitalia slide ♀ 2018–032; RBC.

Material examined: larvae and leafmines (in collection). All collected from *Bistorta officinalis*.

BELGIUM • 100 larvae, 146 mines; Liège, Aldringen, Thommen; 50.22563°N, 6.02781°E; alt. 475 m; 24.vi.2017; WB leg.; SWC. • 2 larvae, 2 mines; Liège, Aldringen, Ulf; 50.2147°N, 6.03125°E; alt. 462 m; 24.vi.2017; WB leg.; SWC. • 16 larvae, 16 mines; Liège, Büllingen, Kleinfüllenbach; 50.39425°N, 6.32834°E; alt. 644 m; 24.vi.2017; WB leg.; SWC. • 3 mines; Liège, Emmels, Deidenberg; 50.31102°N, 6.14507°E; alt. 481 m; 24.vi.2017; WB leg.; SWC. • 3 larvae (used for DNA analysis, 2 destructively), leafmines; Liège, Rocherath, Naturschutzgebiet der Holzwarche; 50.4238°N, 6.314457°E; alt. 585 m; 23.vi.2017; Steve Wullaert leg.; larval slide; RMNH.INS.30919, RMNH.INS.30927, RMNH.INS.30928, RMNH.INS.43338. • 105 mines; same locality data; WB leg.; SWC. • 4 mines; same locality; 19.viii.2017; WB leg.; SWC.

France • several mines; Ardèche, Mt Mézenc, Croix de Boutières; 44.90018°N, 4.18277°E; alt. 1512 m; 29.vii.2009; E.J. van Nieukerken leg.; EventId: EvN no 2009033-K; RMNH.INS.42771. • 3 larvae, many mines; Ardèche, Mt Mézenc, SW slopes; 44.90835°N, 4.18781°E; alt. 1652 m; 29.vii.2009; E.J. van Nieukerken leg.; EventId: EvN no 2009032-M/H/K; RMNH.INS.17942–RMNH.INS.17944 (larvae); RMNH.INS.42667–RMNH.INS.42668 (leafmine samples). • 1 larva, 3 mines; Ardèche, Sainte-Eulalie, Bois des Seigneurs; 44.86449°N, 4.1827°E; alt. 1455 m; 23.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017053-M/H/K; RMNH.INS.31178, RMNH.INS.43887. • many mines; Ardèche, Sainte-Eulalie, Pré du Bois, W of Suc de la Lauzière; 44.84959°N, 4.16323°E; alt. 1469 m; 23.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017052-H/K; RMNH.INS.43472–RMNH.INS.43473. • 2 larvae, mines; Ardèche, Sainte-Eulalie, Suc de la Lauzière, S.; 44.84695°N, 4.17325°E; alt. 1483 m; 23.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017051-M/H.K; RMNH.INS.30932–RMNH.INS.30933, RMNH.INS.43470–RMNH.INS.43471. • 2 larvae, mines; Puy-de-Dôme, St. Alyre-ès-Montagne, Lac de St. Alyre, S. shore; 45.37424°N, 2.94417°E; alt. 1224 m; 14.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017036-M/H/K; RMNH.INS.30929–RMNH.INS.30930, RMNH.INS.43463, RMNH.INS.43465, RMNH.INS.43872. • 8 mines; Puy-de-Dôme, Monts Dore, Chastreix-Sancy, Roc de Courlande, S.; 45.52231°N, 2.7688°E; alt. 1339 m; 13.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017034-H/K; RMNH.INS.43868, RMNH.INS.43869. • 2 larvae, many mines; Puy-de-Dôme, Monts Dore, Chastreix-Sancy, Res. Nat, Fontaine Salée; 45.51214°N, 2.79792°E; alt. 1338 m; 13.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017035-M/K/H; RMNH.INS.30931, RMNH.INS.30939; RMNH.INS.43462, RMNH.INS.43870, RMNH.INS.43871. • 1 larva, several mines; Puy-de-Dôme, Monts Dore, Col de la Croix de St Robert, SE, along road; 45.55873°N, 2.84282°E; alt. 1416 m; 16.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017039-K; RMNH.INS.31004, RMNH.INS.43466, RMNH.INS.43875. • 2 mines; Puy-de-Dôme, Monts Dore, Vallée de Chaudefour, Res. Nat., upper valley E; 45.52916°N, 2.83496°E; alt. 1346 m; 16.vii.2017; E.J. van Nieukerken leg.; EventId: EvN no 2017037-K; RMNH.INS.43873.

Poland • 29 larvae [ethanol, some destructively extracted, larval slide], Dubienka; 51.00998°N, 23.92238°E; alt. 175 m; 02.vii.2004; K. Pałka leg.; EventId: EvN no 2004905-M; RMNH.INS.11854, RMNH.INS.11855, RMNH.INS.12423. • 15 larvae [ethanol, some destructively extracted], Poleski Nat. Park: Bagno Bubnów; 50.94514°N, 22.637°E; alt. 287 m; 01.vii.2004; K. Pałka leg.; EventId: EvN no 2004904-M; RMNH.INS.11852, RMNH.INS.11853.

Switzerland • 5 larvae, Bern, Cortébert; 47.15431°N, 7.12766°E; alt. 1260 m; 04.viii.2017; R. Bryner leg.; RBC. • leafmines; Bern, Saicourt; 47.2555°N, 7.16569°E; alt. 920 m; 02.viii.2017; R. Bryner leg.; RBC. • leafmines; Bern, St-Imier; 47.17653°N, 6.94997°E; alt. 1000 m; 02.viii.2017; R. Bryner leg.; RBC. • leafmines; Bern, Tramelan; 47.24232°N, 7.06144°E; alt. 1010 m; 04.viii.2017; R. Bryner leg.; RBC. • leafmines; Jura, Lajoux; 47.28891°N, 7.1292°E; alt. 930 m; 04.viii.2017; R. Bryner leg.; RBC. • leafmines; Jura, Le Bémont; 47.25269°N, 7.02997°E; alt. 970 m; 04.viii.2017; R. Bryner leg.; RBC. • leafmines; Jura, Montfaucon; 47.2819°N, 7.08989°E; alt. 850 m; 04.viii.2017; R. Bryner leg.; RBC. • leafmines; Neuchâtel, La Brévine; 46.97567°N, 6.58592°E; alt. 1030 m; 05.viii.2017; R. Bryner leg.; RBC. • leafmines; Neuchâtel, Le Cerneux-Péquinot; 47.00417°N, 6.66141°E; alt. 1060 m; 05.viii.2017; R. Bryner leg.; RBC. • leafmines; Vaud, Ste-Croix; 46.84122°N, 6.47154°E; alt. 1090 m; 05.viii.2017; R. Bryner leg.; RBC.

Additional online observations. All observed on *Bistorta officinalis*.

Belgium • 8 larvae, 30 mines; Liège, Butchenbach, Schwalm; 19.vii.2018; leg. Evert Van de Schoot & Ward Tamsyn; 50.4980, 6.2562; https://waarnemingen.be/observation/160366513/. • 1 larva, 10 mines; Liège, Butchenbach; Schwalm; 19.vii.2018; leg. Evert Van de Schoot; 50.4898, 6.2714; https://waarnemingen.be/observation/160463202/. • 3 mines; Liège, Malmedy, Fagne de la Polleur; 22.vii.2017; leg. Wim Declercq; 50.5105, 6.0744; https://waarnemingen.be/observation/141930607/. • 13 larvae, 16 mines; Liège, Rocherath, Vallée de la Holzwarche; 24.vi.2017; leg. WB; 50.3961°N, 6.3318°E; https://waarnemingen.be/observation/140955240/. • 2 larvae, 2 mines; same locality and date; leg. Chris Steeman & Ben Steeman; 50.4151°N, 6.3283°E; https://waarnemingen.be/observation/140964609/. • 1 mine; same locality; 22.vii.2017; leg. Wim Declercq; 50.4172°N, 6.3268°E; https://waarnemingen.be/observation/141930615/. • 2 larvae, 2 mines; same locality; 22.vii.2017; leg. Wim Declercq; 50.4240°N, 6.3145°E; https://waarnemingen.be/obser-

vation/141930621/. • 1 mines; same locality; 14.vii.2018; leg. Jurgen Dewolf & Ruben Recour; 50.4237°N, 6.3147°E; https://waarnemingen.be/observation/159940474/. • 20 larvae, 40 mines; same locality; 23.vii.2018; leg. Wouter Mertens; 50.4209°N, 6.3199°E; https://waarnemingen.be/observation/160514341/. • 14 larvae, 34 mines; same locality; 23.vii.2018; leg. Chris Steeman ; 50.4237°N, 6.3145°E; https://waarnemingen.be/observation/160831314/. • 4 larvae, 25 mines; same locality; 29.vii.2018; leg. Regis Nossent ; 50.4236°N, 6.3146°E; https://waarnemingen.be/observation/160702446/. • 1 larva, 1 mines; same locality; 06.ix.2018; leg. WB; 50.4233°N, 6.3141°E; https://waarnemingen.be/observation/162347416/. • 2 larvae, 7 mines; same locality; 08.viii.2019; leg. WB; 50.4233°N, 6.3152°E; https://waarnemingen.be/observation/178051781/. • 16 larvae, 26 mines; same locality; 10.viii.2019; leg. WB; 50.4209°N, 6.3182°E; https://waarnemingen.be/observation/179079003/. • 1 larva, 8 mines; same locality; 08.viii.2020; leg. WB; 50.4246°N, 6.3145°E; https://waarnemingen.be/observation/203156253/. • 19 larvae, 76 mines; Liège, Waimes, Rue du Poncé; 24.vii.2018; leg. Chris Steeman & Steve Wullaert; 50.4387°N, 6.1095°E; https://waarnemingen.be/observation/160817401/. • 3 larvae, 4 mines; same locality; 09.viii.2020; leg. Eric Wille; 50.4384°N, 6.1093°E; https://waarnemingen.be/observation/198140856/. • 14 larvae, 15 mines; Luxembourg, Vance, Marais de Vance; 02.vii.2017; leg. Wim Declercq; 49.6719°N, 5.6807°E; https://waarnemingen.be/observation/141082788/. • 7 larvae, 8 mines; Luxembourg, Volaiville, Vallée de la Sûre; 20.vi.2020; leg. WB; 49.8658°N, 5.6846°E; https://waarnemingen.be/observation/194673026/.

FRANCE • 1 vacated mine; Jura, Les Rousses; 08.viii.2020; leg. Wim Declercq; 46.4963°N, 6.0759°E; https://observation.org/observation/199070630/.

Discussion

Hostplant

We follow the databases of POWO (2017–2021) and IPNI (2021) in treating *Bistorta* as a separate genus, even though several floras still include it in *Persicaria*. The monophyly of *Bistorta* is well supported by several molecular studies (Kim and Donoghue 2008; Fan et al. 2013; Schuster et al. 2015). Several of the other Lepidoptera that feed on *B. officinalis* (see below) also feed on the boreo-alpine *Bistorta vivipara* (L.) Delarbre. It is possible that *A. ludwigi* could feed on this species as well, although often the leaves are probably too narrow for the mines. In Japan *B. vivipara* is host for another, unnamed species of *Antispilina* (Lee and Hirowatari 2013).

Phylogeny

In both phylogenetic studies the sister-group relationship between *Antispilina* Hering, 1941 and *Holocacista* Walsingham & Durrant, 1909 is well supported (Milla et al. 2017, 2019). Together these genera are sister to the New World clade *Coptodisca* Walsingham, 1895 plus *Aspilanta* van Nieukerken & Eiseman, 2020, and all these share the reduced venation that is here also observed in *A. ludwigi*. In many morphological details *Antispilina* and *Holocacista* are rather similar, but the absence of an epiphysis and the reduction of the labial palpi to two articles are characteristic for *Antispilina*. The last character together with the hostplant specialisation are considered to be synapomorphies for this genus (van Nieukerken and Eiseman 2020).

Conservation

The habitat where this species occurs, nutrient-poor wet meadows, is much threatened in Europe. Two butterflies feeding on *Bistorta officinalis*, viz. *Boloria eunomia* (Esper, 1799) and *Lycaena helle* (Denis & Schiffermüller, 1775) are amongst the locally most threatened butterflies, although they still have a large distribution area covering most of northern Asia (Swaay and Warren 1999; Gorbunov

2001; Kudrna 2002). *Lycaena helle* occurs on each of the eastern Polish localities of *A. ludwigi*, whereas *B. eunomia* is more local and occurs only in Bagno Bubnów and Dubienka (Anna Mazurkiewicz, personal communication). Since *A. ludwigi* has not been found in Germany in recent years, we have no information on its sympatric occurrence with these butterflies, both of which are very rare and threatened in Germany, *L. helle* is critically endangered, it has disappeared from many former localities due to afforestation and drying of its habitats (Pretscher 1998; Settele et al. 2000).

In Belgium (Wallonia) most finds of *A. ludwigi* are in the Holzwarche valley in the Haute Fagnes area in Rocherath. The Holzwarche valley is very extensive and stretches from the plateau of Losheimergraben to Lake Bütchenbach. Here *Lycaena helle* occurs on almost every location were *A. ludwigi* was found. *Boloria eunomia* has about the same distribution in Belgium as *L. helle*, but is slightly commoner. Both species have a stronghold in the Holzwarche area where the foodplant is abundant (Waarnemingen.be 2020). *Lycaena helle* is considered vulnerable in Wallonia, where it is legally protected since 2001; a Life+ project launched in 2009 in Southern Belgium aimed at restoring its habitats. The objective of the project was to restore and manage at least 250 ha in function of *L. helle* (Goffart et al. 2014). Since the start of this project the online platform "waarnemingen.be" shows increasing numbers of observations for both *L. helle* and *B. eunomia* since 2009. This progress ensures that the host plant is preserved in most areas that are also good for *A. ludwigi* and that this species has the opportunity to expand.

In the Massif Centrale in France, where we found *A. ludwigi* in many places where we checked the hostplant, *L. helle* still has many populations (Muséum national d'Histoire naturelle 2003–2021; Habel et al. 2011; Merlet and Houard 2012), but *B. eunomia* does not occur there (Muséum national d'Histoire naturelle 2003–2021). In the Swiss Jura *A. ludwigi* was found in all visited sites of *L. helle*, and likewise in a locality in the northern Alps.

Other Lepidoptera species feeding almost exclusively on *Bistorta officinalis* are the rare alpine *Boloria titania* (Esper, 1781), and the micromoths *Coleophora pratella* Zeller, 1871 and *Phiaris astrana* (Guenée, 1845). The last species, so far only known from the French and Swiss Jura (Muséum national d'Histoire naturelle 2003–2021; Lepiforum e.V. 2008–2021; SwissLepTeam 2010), also occurs in several of the Swiss localities of *A. ludwigi*, including that in Fig. 37.

In general, we urge lepidopterists who monitor the afore-mentioned butterflies to check the hostplants for the conspicuous leafmines and post photographs of these online in observation platforms. Monitoring these mines is relatively easy, can be done even under poor weather conditions, and contributes to the knowledge of the biodiversity of these valuable habitats.

As *Lycaena helle* is the most common companion species, it is likely that management for that species also will benefit *A. ludwigi*. Most important is a low-intensity management, very light grazing in a rotational way, avoiding the sensitive periods of the butterfly (and moth) April-July, and preventing natural reforestation (Swaay et al. 2012). For populations of *A. ludwigi* at higher elevations, the period to avoid management should perhaps be extended to the first half of August, when there are still larvae in the mines.

Distribution

After we learned about the habitat of this species, it was easy to find it in many places where it had not been previously recorded. This was a clear indication that *Antispilina ludwigi* was overlooked, despite its conspicuous leafmines. Many lepidopterists still collect or observe mostly focusing on adults with artificial lights or by netting, whereas collecting and photographing of larvae and

leafmines is rarely done in many parts of Europe, despite an increasing interest in leafmine studies, especially in the British Isles, the Netherlands and Belgium (Edmunds 2020; Ellis 2020; Wullaert 2021). As the hostplant *Bistorta officinalis* has a wide distribution in Europe and throughout Siberia, we expect that *Antispilina ludwigi* can be found in many more sites in Europe and even northern Asia. In East and South Asia several other species of *Antispilina* have been found, feeding on other species of Polygonaceae, which will be described elsewhere (Lee and Hirowatari 2013; Milla et al. 2017). Focussed searching in localities where *Lycaena helle* is known will probably be the easiest way to discover new populations. The new records suggest a wider distribution in the European mountains, and the species should particularly be searched in German mountains, other parts of France, Austria, northern Italy, Slovenia, Romania, Belarus, Ukraine and the European part of Russia.

Our results also stress that focused searching on host plants of poorly known Lepidoptera and other insects may be more successful than anticipated. We observed a similar result in the rare leafminer *Digitivalva arnicella* (Heyden, 1863) (Glyphipterigidae) on *Arnica montana* in the Netherlands and Belgium (van Nieukerken and Koster 1999; Wullaert 2019).

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